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A hypomyelinating leukodystrophy in German Shepherd dogs

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Abstract

Background: Shaking puppy syndrome is commonly attributed to abnormal myelination of the central nervous system.

Hypothesis/Objectives: To report the long-term clinical course and the imaging characteristics of hypomyelinating leukodystrophy in German Shepherd dogs.

Animals and Methods: Three related litters with 11 affected dogs.

Results: The 11 affected dogs experienced coarse, side-to-side tremors of the head and trunk, which interfered with normal goal-oriented movements and disappeared at rest. Signs were noticed shortly after birth. Nine dogs were euthanized, 3 dogs underwent pathological examination, and 2 littermates were raised by their breeder. Tremors improved gradually until 6 to 7 months of age. Adult dogs walked with severe residual pelvic limb ataxia. One dog developed epilepsy with tonic-clonic seizures at 15 months of age. Conventional magnetic resonance imaging (MRI) disclosed homogenous hyperintense signal of the entire subcortical white matter in 3 affected 7-week-old dogs and a hypointense signal in a presumably unaffected littermate. Subcortical white matter appeared isointense to gray matter at 15 and 27 weeks of age on repeated MRI. Abnormal white matter signal with failure to display normal gray-white matter contrast persisted into adulthood. Cerebellar arbor vitae was not visible at any time point. Clinical signs, MRI findings, and pathological examinations were indicative of a hypomyelinating leukodystrophy. All parents of the affected litters shared a common ancestor and relatedness of the puppies suggested an autosomal recessive mode of inheritance.

Conclusion: We describe a novel hypomyelinating leukodystrophy in German Shepherd dogs with a suspected inherited origin.

KEYWORDS

animal model, brain maturation, development, dysmyelination, genetic, hypomyelination, inherited, leukoencephalopathy, seizures, tremor, white matter

Abbreviations: BAER, brainstem auditory evoked response; CNS, central nervous system; CT, computed tomography; FLAIR, fluid attenuation inversion recovery; MRI, magnetic resonance imaging; NCV, nerve conduction velocity; PMD, Pelizaeus Merzbacher disease; STIR, short tau inversion recovery.

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1 | INTRODUCTION

Leukodystrophies in humans are a group of heritable disorders affecting the white matter of the central nervous system (CNS) with glial cell or myelin sheath abnormalities, characteristic magnetic resonance imaging (MRI) features, and lack of systemic manifestations.¹ Until recently, it has been challenging to classify leukodystrophies because of their variable presentation and heterogeneous genetic origins. Leukodystrophies may be further classified based on pathological changes and pathophysiologic mechanisms derived from gene discovery. These myelin disorders can present as hypomyelination, demyelination, vacuolization, astrocytopathy, leuko-axonopathy, microgliopathy, and leuko-vasculopathy.²

In veterinary patients, hypomyelination and dysmyelination (delayed myelination) of the CNS commonly are associated with a shaking puppy syndrome. Characteristic findings are typical coarse tremors of the head and body, which may improve with time. Signs are present since birth or noticed in 2- to 3-week-old puppies before weaning. The subsequent clinical course differs among breeds, scientific reports, and the myelination defect present.³ Samoyeds,⁴ Bernese Mountain dogs,⁵ Welsh Springer Spaniels,⁶ Weimaraners,^{7,8} Lurcher dogs,⁹ and Chow-Chows^{10,11} are affected breeds. Genetic testing is available for Springer Spaniels (proteolipid protein gene, *PLP1*; X-linked recessive)¹² and Weimaraners (folliculin-interacting protein 2, *FNIP2*; autosomal recessive),¹¹ and a synergistic gene mutation has been suspected in Chow-Chows.¹¹ Overlapping clinical features exist in spongiform leukoencephalomyelopathy in Border Terriers,^{13,14} in Belgian Shepherd dogs with progressive spongy degeneration and cerebellar signs (*KCNJ10*¹⁵; *ATP1B2*¹⁶), Standard Schnauzers with leukodystrophy (*TSEN54*¹⁷), and Cretan Hund leukoencephalopathy¹⁸ associated with parvovirus infection. Border terriers with spongiform leukoencephalomyelopathy also show severe generalized coarse body tremors resulting in swinging side-to-side or rocking horse movements and cerebellar ataxia at 15 to 28 days of age.^{13,14} Belgian Shepherds with spongiform degeneration and *ATP1B2* gene variant show progressive signs including ataxia, seizures, central blindness, pacing, and circling leading to euthanasia between 6 and 17 weeks of age.¹⁶ The clinical course remains poorly defined in many dog breeds, and complete recovery, incomplete recovery with remaining disabilities or early euthanasia may occur.^{4-7,10,14} The genetic leukodystrophies have a characteristic appearance on MRI.^{19,20} Description of the abnormal MRI patterns has become a valuable part of phenotyping of suspected inherited diseases of the CNS and specifically the leukodystrophies in dogs²¹⁻²³ and humans.^{19,20} There is a lack of description of the MRI features of hypomyelinating leukodystrophies in the veterinary literature.^{24,25}

We describe a shaking puppy syndrome in German Shepherd dogs with a hypomyelinating leukodystrophy. Our aim was to describe the clinical course, long-term follow-up and MRI characteristics, to identify additional cases, and provide adequate counseling of breeders and owners of affected dogs.

2 | MATERIAL AND METHODS

A German breeder of German Shepherd dogs contacted a geneticist (T. Leeb) for investigation of a shaking puppy syndrome. Subsequently,

additional cases were identified. In total, 3 affected litters (2 from Germany and 1 from Finland) were reported.

Dogs were studied by video footage provided by the breeder and caretakers, clinical examination, neurological examination, laboratory examination, and advanced imaging at 7 weeks of age (brain MRI, 4 dogs; spine MRI, 1 dog; computed tomography [CT] angiography, 1 dog). Two dogs were followed until 3 years of age, and in 1 of these dogs repeated brain MRI was acquired (15, 27, and 69 weeks of age). One dog from each affected litter underwent necropsy examination.

Laboratory evaluation included routine hematology and serum biochemistry profile, serum electrolyte concentrations, creatine kinase activity, fasted and postprandial blood ammonia concentrations, ammonia tolerance testing, bile acid concentrations, blood gas analysis, blood lactate concentrations, serum cobalamin concentrations (Synlab, Germany), urinalysis, and screening for inherited neurometabolic disorders (urinary organic acids, oligosaccharides and mucopolysaccharides; plasma amino acids; Biocontrol, Germany).

Routine brain MRI (1.5T MAGNETOM Symphony, Siemens, Erlangen, Germany) included the following sequences: T2-weighted turbo spin echo (T2W), T1-weighted spin echo (T1W) before and after bolus administration of contrast agent (Omniscan TM, GE Healthcare; gadodiamide, 0.3 mL/kg IV), T2-weighted fluid attenuation inversion recovery (FLAIR), and T2-weighted short tau inversion recovery (STIR) images. Subcortical white matter (internal capsule, corona radiata, centrum semiovale, corpus callosum), cerebellar white matter, and cerebellar peduncles were compared to gray matter of the cerebral and cerebellar cortex in all sequences, and also compared to an clinically unaffected littermate and to normal brain maturation in Beagles.²⁶ The T2W midsagittal and transverse brain images of dog 3 at 69 weeks of age were compared to brain images from 3 control dogs with no clinical signs of leukodystrophy (5-month-old male Rhodesian Ridgeback, 13-month-old female Rhodesian Ridgeback, 4-month-old male Labrador Retriever). Size of the caudal cranial fossa was assessed by caudal fossa ratio, and cerebellar size with cerebellar index.^{27,28} All data were expressed as the mean of 2 measurements, and all images were evaluated by consensus (A. Brühnschwein, A. Fischer, P.R. Quitt). DicomPACS viewer software version 8.3.15 (Oehm und Rehbein GmbH, Rostock, Germany) was used for image review and calculations. The spinal cord was imaged in dog 3. All sequence parameters are given in Table S1. Computed tomographic angiography (Multislice CT-Scanner, Somatom Definition AS, Siemens Healthineers, Erlangen, Germany) of the liver (Accupaque TM 300, GE Healthcare; iohexol 2 mL/kg IV) was performed in 1 dog to exclude a portosystemic shunt. Electrodiagnostic examination (electromyography, brainstem auditory evoked response [BAER], nerve conduction velocity [NCV]; Viking Quest; Nicolet, Germany) was performed in 1 dog after routine procedures. All investigations were performed under general anesthesia induced with propofol (Narcofol, Cp-pharma; repeated 1 mg/kg IV) and maintained with isoflurane after premedication with butorphanol (Dolorex, MSD Animal Health, 0.3 mg/kg) and diazepam (Ziapam, Ecuphar, 0.3 mg/kg). Three affected dogs, 1 from each litter, were subjected to complete necropsy examination at 3, 5, and 7 weeks of age. On necropsy, brain, spinal cord, and associated nerve roots were harvested using an extensive

craniectomy-laminectomy approach. Tissues were immediately immersed in 10% neutral buffered formalin for routine formalin-fixed paraffin-embedded tissue histology. Additional samples were taken freshly from subcortical white matter, capsula interna, corpus medullare of cerebellar roof, and spinal cord and fixed in 2.5% glutaraldehyde in 0.1 M Soerensen's phosphate buffer for epoxy embedding and electron microscopy. The formalin-fixed brain was dissected and sampled in accordance with international veterinary epilepsy task force guidelines.²⁹ Tissue sections underwent automatic procession, paraffin embedding, and cutting at 5 μ m slice thickness. The sections were stained with hematoxylin-eosin, Wölcke-Spielmeyer-Schröder impregnation technique, and Klüver-Barrera's luxol fast blue-cresyl echt violet. Semithin scout sections were stained with azure II methylene blue-safranin O. Selected zones were trimmed, ultracut at 50 nm, and contrasted with uranyl acetate and lead citrate.

3 | RESULTS

Eleven German Shepherd dogs (9 males, 2 females) with shaking puppy syndrome were identified (Table S2). The dogs originated from Germany (2 litters) and Finland (1 litter). The breeders recognized the abnormal movements in the second week of life. The shaking puppy phenotype was confirmed by review of video footage of all litters. No difference in size or severity of clinical signs between affected male and female dogs was noted. Eight affected dogs were euthanized at 3, 5, or 7 weeks of age. The breeder of the most recent litter decided on further investigations and provided intensive care until the dogs were able to feed and maintain themselves. This enabled us to monitor the long-term clinical course in 2 affected male dogs from this litter.

3.1 | Clinical course

The dogs showed gradual and slow improvement but failed to recover completely. At 4 months of age, the dogs were only able to walk for several meters and showed severe hypermetric pelvic limb ataxia and paresis. The adult dogs walked with residual pelvic limb ataxia. One dog developed epilepsy.

3.2 | Early clinical course (11 days-16 weeks)

3.2.1 | Eleven days

The breeder noted neurological signs for the first time. Three male puppies showed coarse 2 to 3 Hz side-to-side tremors of the hindquarters spreading to the neck and also involving the head. At this age, affected and unaffected dogs from this litter were only able to crawl and experienced falling and rolling. There was no difference in size or weight between affected and unaffected dogs (Video S1).

3.2.2 | Seventeen days

The difference between unaffected and affected puppies became obvious. The 3 shaking puppies were only able to crawl, showed pronounced coarse side-to-side tremors in the hindquarters (2-3 Hz) and coarse tremors of the head and neck. Unaffected littermates showed no tremors, were able to walk a few steps, moved the head more purposefully (eg, for sniffing), and showed explorative behavior (Video S2).

3.2.3 | Four weeks

The affected puppies were able to stand but unable to walk without falling. They still required much support with hand feeding. The trunk was affected by up-and-down bouncing movements whereas head tremors were side-to-side with a rotatory component. Movements continued when the dogs were lying sternally and resolved in lateral recumbency, during rest and sleep. Neurological examination indicated normal mentation, decreased pelvic limb postural reactions, and absent menace response (which was considered normal for the dogs' age). The dogs experienced episodes of intermittent saccadic oscillation of both eyes with multidirectional aberrant eye movement without a drifting phase. In the unaffected littermates, gait was considered normal for the age of the dogs. No tremors or shakes were recognized, pelvic limb postural reactions were mildly delayed. No difference in size and weight were detected between affected and unaffected littermates, and unaffected dogs appeared to develop normally (Video S3).

3.2.4 | Seven weeks

Affected puppies could stand and walk a few steps without support, but fell to the side intermittently. A side-to-side and up-and-down whole-body tremor was still present. No intension tremor was evident during eating. Neurological examination indicated normal mentation, absent menace response, and saccadic oscillations. Postural reactions were absent in the pelvic limbs and delayed in the thoracic limbs with decreased spinal reflexes (Video S4).

3.2.5 | Nine weeks

Affected puppies could walk a few steps without support, but still fell to the side intermittently. Whole body shakes were still obvious. Movement and stability of the thoracic limbs appeared improved. Wide-based stance of the thoracic limbs and pronounced ataxia and dragging of the pelvic limbs with "bunny hopping" occurred (Video S4).

3.2.6 | Twelve weeks

The dogs were able to stand and walk 1 to 2 m with pronounced ataxia and paresis of pelvic limbs and slightly hypermetric thoracic

limbs. On some occasions, the puppies collapsed and dragged the pelvic limbs. Tremors of the head had almost disappeared, but coarse side-to-side tremors of the hindquarters were still obvious in the standing dogs, although less severe (Video S5).

3.2.7 | Sixteen weeks

The dogs now were able to walk short distances. Gait was characterized by severe pelvic limb ataxia and paresis with hypermetria, intermittent hopping, and dragging of the limbs. Head tremors had subsided. Coarse tremors of the trunk and back had further diminished and were less obvious (Video S5).

3.3 | Long-term clinical course (6 months-3 years)

The 6- to 7-month-old dogs were able to walk longer distances (up to 500 m), but continued to experience obvious pelvic limb ataxia and paresis. Mild truncal tremor was still evident. At 10 months of age, further improvement was noted. The dogs now were able to walk longer distances and run. Tremors were no longer evident and further improvement of ataxia was noted. Residual pelvic limb ataxia was still obvious when the dogs were walking slowly (Video S6).

One dog presented at 15 months of age because of a recent onset of focal motor (lip smacking, chewing, salivation) and tonic-clonic seizures (dog 3). Seizures occurred predominantly during activity and required treatment with several anti-seizure drugs (phenobarbital, potassium bromide, levetiracetam) for adequate control. Laboratory examination (CBC, serum biochemical profile, ammonia tolerance test, blood lactate concentration) and cerebrospinal fluid analysis were normal. Abnormal white matter signal intensity was still evident on T2W brain MRI. Subsequently, pelvic limb ataxia worsened in this dog requiring support with a cart, and was attributed to the adverse effects of anti-seizure drugs, especially bromide. After having 2 seizures per month for a period of 7 months, dog 3 became seizure-free and no further epileptic seizures occurred. A tapered dosage of bromide was administered. In the other dog (dog 2), no change in residual pelvic limb ataxia occurred until 3 years of age (Video S7).

3.4 | Magnetic resonance imaging

Brain MRI showed homogenous hyperintense appearance of the entire subcortical white matter at 7 weeks of age on T2W, FLAIR images in affected dogs (Figures 1 and 2). A similar pattern was seen on STIR images (Figure S1). The corpus callosum and cerebellar peduncles appeared isointense to gray matter and there was poor differentiation between cerebellar gray and white matter contrast on midsagittal views. In contrast, MRI of the presumably unaffected 7-week-old female littermate showed unremarkable hypointense signal of the subcortical white matter, corpus callosum, and cerebellar peduncles. There was clear distinction between cerebellar gray and

white matter, resulting in good visibility of the cerebellar arbor vitae (Figure 1E). Pre- and postcontrast T1W brain images were normal. Subcortical white matter had mild hyperintensity compared to gray matter in all dogs at 7 weeks of age on T1W images. There was no evidence of ventricular enlargement. Cerebellar and caudal fossa size was within published reference ranges and did not differ between affected dogs and the unaffected littermate at 7 weeks of age (Tables S3 and S4).^{26,27} The spinal cord was imaged in 1 affected dog and was normal in appearance.

Follow-up brain MRI at 15 and 27 weeks of age in 1 affected dog showed abnormal isointense appearance of subcortical white matter on T2W and FLAIR images (dog 3, Figures 3B,D and 4A,C). Thus,

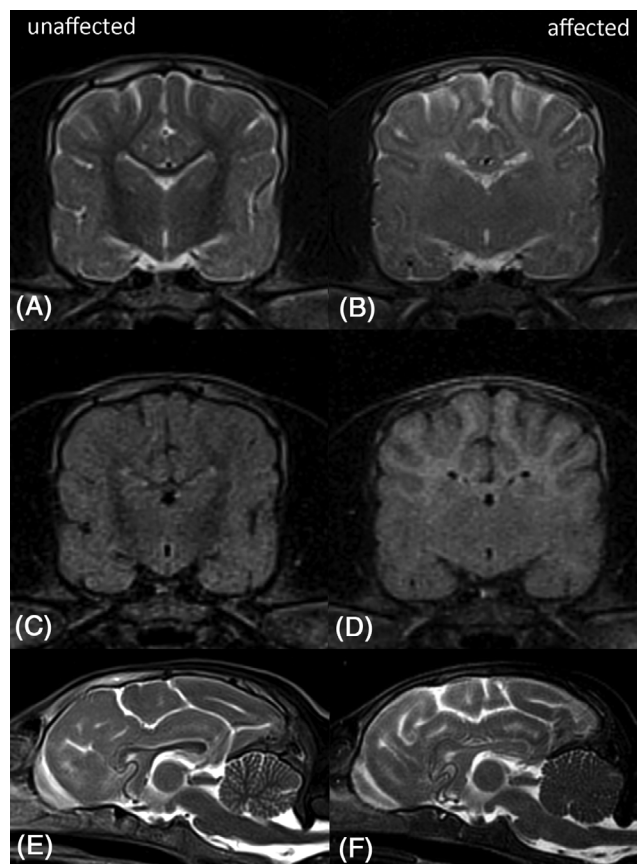


FIGURE 1 Brain MRI at 7 weeks of age. The figure displays MRI from the unaffected and 1 affected littermate (dog 2) at 7 weeks of age. The upper row shows T2W transverse images (A, B) at the level of the interthalamic adhesion, the middle row shows corresponding transverse FLAIR images (C, D), and the lower row shows T2W midsagittal images (E, F). Subcortical white matter appears hypointense compared to gray matter in the unaffected littermate. In contrast, subcortical white matter appears homogeneously hyperintense in the affected littermate. Corpus callosum appears hypointense to gray matter in the unaffected littermate (E) and isointense in the affected dog (F). The affected dog shows poor differentiation between cerebellar gray and white matter and few hyperintense patches in the brainstem on midsagittal T2W images (F). FLAIR, fluid attenuation inversion recovery; MRI, magnetic resonance imaging

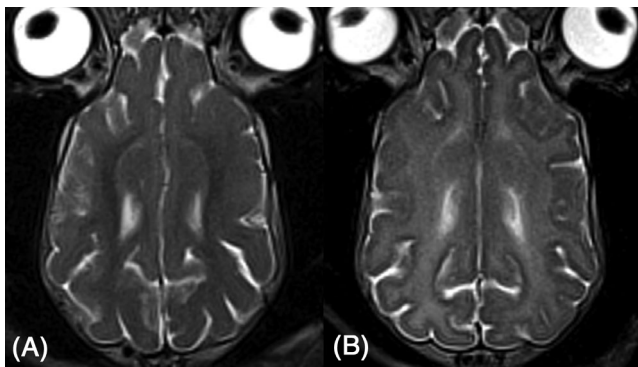


FIGURE 2 T2W dorsal brain MRI at 7 weeks of age. The figure displays dorsal T2W images of the brain from the unaffected dog and 1 affected dog at 7 weeks of age. Subcortical white matter appears homogeneously hypointense to gray matter in the unaffected littermate (A) and hyperintense in the affected dog (B). MRI, magnetic resonance imaging

white matter signal intensity on T2W and FLAIR images resembled the T2W isointense transition phase of 6-week-old dogs.²⁶ The STIR images at 27 weeks of age showed hypointense white matter as described in the normal maturation phase in Beagles.²⁶

On follow-up MRI at 69 weeks of age, the subcortical and cerebellar white matter still appeared isointense or only mildly hypointense to gray matter (dog 3, Figure 4). Gray-white matter contrast was decreased in the cerebrum, and the cerebellar arbor vitae was not visible when compared to a control dog (Figure 5).

3.5 | Other investigations

All laboratory examinations were unremarkable except for a borderline increase in blood ammonia concentration in affected puppies, which normalized in the adult dogs. Ammonia concentrations were in the upper end of the reference range in the unaffected littermate. Bile acid concentrations and neurometabolic screening, as well as serum cobalamin and lactate concentrations, were normal. Oral ammonia tolerance tests at 27 weeks of age were normal (dogs 2 and 3). Ophthalmic examinations were normal (dogs 2 and 3). Electrodiagnostic testing of 1 dog (dog 3) at 27 weeks of age identified spontaneous electrical activity (2+) in the infraspinatus muscle. Tibial motor NCV and BAER were unremarkable. Computed tomographic angiography showed normal vascularization of the liver at 7 weeks of age in 1 dog.

3.6 | Pathology

Three dogs, 1 from each litter, underwent pathological examination. No gross or histopathological changes were identified outside of the neuroaxis. Both German dogs presented with diffusely diminished myelin staining predominantly in subcortical white matter of the forebrain, corpus callosum, semiovale center, and foliary white matter and corpus

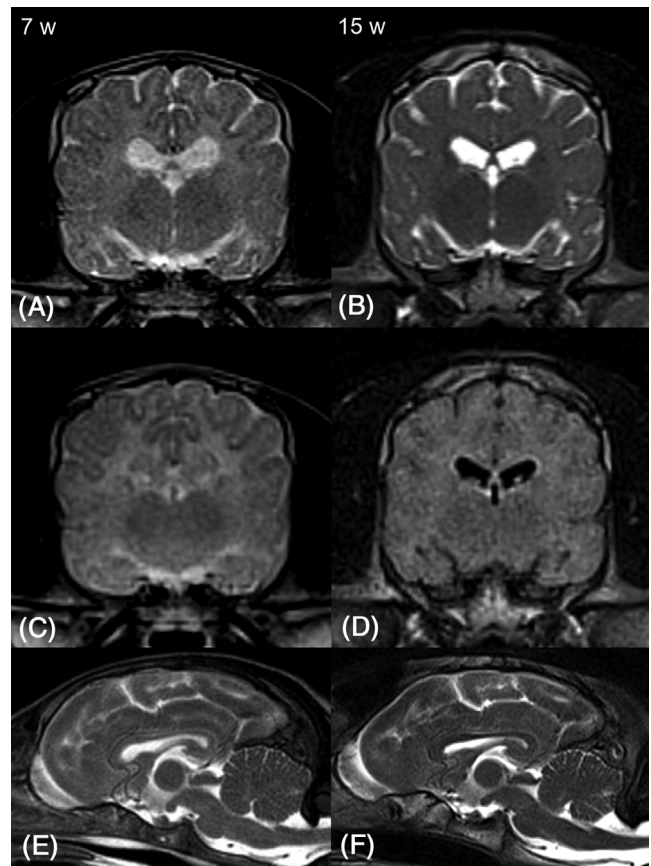


FIGURE 3 Follow-up brain MRI at 15 weeks of age. The figure displays MRI of the brain from the affected dog 3 at 7 and 15 weeks of age. The upper row shows T2W transverse images at the level of the interthalamic adhesion (A, B), the middle row shows corresponding transverse FLAIR images (C, D), and the lower row shows T2W midsagittal images (E, F). Subcortical white matter appears hyperintense to gray matter at 7 weeks and isointense at 15 weeks of age on T2W and FLAIR images. Thus the affected dog failed to display the normal transition phase to white matter hypointensity, which occurs in healthy Beagles at 6 weeks (T2W) and 8 to 36 weeks of age (FLAIR).²⁶ FLAIR sequence failed in this dog at 7 weeks of age presumably due to artifact (C).³⁰ FLAIR, fluid attenuation inversion recovery; MRI, magnetic resonance imaging

medullare of the cerebellum (Figure 6). This finding corresponded to disproportionately thin myelin sheaths (compared to a 7-week-old age-matched control) on semithin sections (Figure 6H,I) and abundance of hypomyelinated axons, dilated myelin tubes and occasional myelin splitting on transmission electron microscopy. Some areas also had rare intracellular degenerate myelin figures. Hypomyelination was accompanied by moderate to marked diffuse astroglial and oligodendroglial hypercellularity (Figure 6). The white matter of the internal capsule, ascending and descending white matter tracts, and rostral commissure were similarly but less extensively affected. No such changes were seen in peripheral nerves. In addition to the hypomyelination, the number of white matter neurons (ie, interstitial neurons) of the forebrain subjectively appeared mildly increased for a 7-week-old dog. Linear edema and neuropil vacuolation were present along the inner laminae of the cerebral cortex. This finding corresponded to interlamellar splitting.

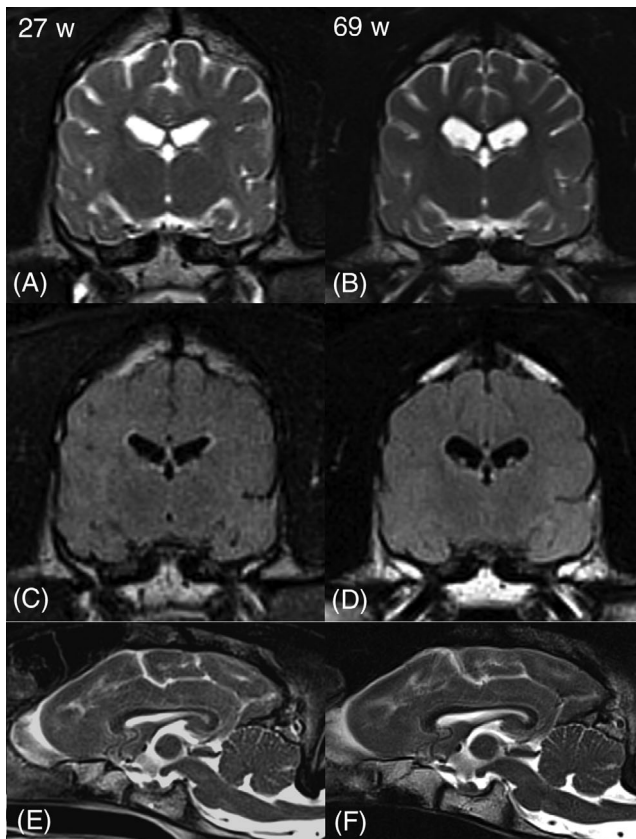


FIGURE 4 Follow-up brain MRI at 27 and 69 weeks of age. The figure displays MRI of the brain from the affected dog 3 at 27 and 69 weeks of age. The upper row shows T2W transverse images at the level of the interthalamic adhesion (A, B), the middle row shows corresponding transverse FLAIR images (C, D), and the lower row shows T2W midsagittal images (E, F). Subcortical white matter appears isointense to gray matter at 27 weeks of age on T2W and FLAIR images (A, C). In the adult dog, at 69 weeks of age, the subcortical (B, D) and cerebellar white matter still appeared isointense to mildly hypointense without normal gray-white matter contrast (F). The cerebellar arbor vitae is not discernible (E, F). On midsagittal images, the corpus callosum appears more hypointense at 27 and 69 weeks of age (E, F). FLAIR, fluid attenuation inversion recovery; MRI, magnetic resonance imaging

Similar findings were seen on necropsy of the Finnish dog (litter 3) where severe diffuse pallor consistent with hypomyelination and mild multifocal vacuolar degeneration were seen in the caudal brainstem and spinal cord. They had no other gross abnormalities, but 1 dog had signs of mild multifocal interstitial pneumonia of unknown origin (no lesions consistent with any infectious agent were observed) with mild diffuse leukostasis.

3.7 | Genetics

Pedigree analysis showed 2 closely related litters from Germany, which shared a common ancestor with the affected Finnish litter (Figure 7). An autosomal recessive trait was suspected with both

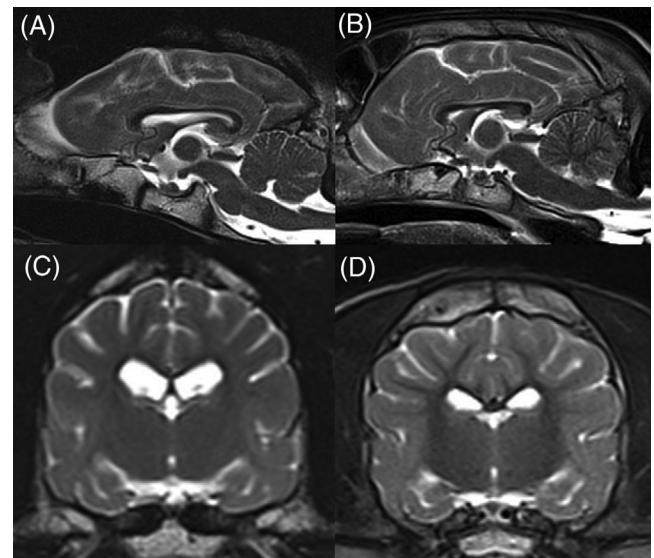


FIGURE 5 Brain MRI from one adult affected dog and a control dog. The figure displays T2W MRI from one affected dog (A, C) at 69 weeks of age (dog 3), and a control dog (B, D; 4-month-old). The upper row shows T2W midsagittal images at the level of the interthalamic adhesion (A, B) and the lower row shows the corresponding transverse images (C, D). The affected adult dog fails to display normal gray-white-matter contrast in the cerebrum (C) and cerebellum (A). The cerebellar arbor vitae is not visible on midsagittal images (A). The entire subcortical and cerebellar white matter appears isointense to mildly hypointense compared to gray matter. The control dog shows prominent hypointensity of the subcortical white matter (D) and a clearly visible cerebellar arbor vitae (B). MRI, magnetic resonance imaging

sexes, female and male dogs, affected. Whole genome sequencing was performed on genomic DNA isolated from whole blood of 1 affected dog, 9 German Shepherd controls, and 580 other canine genomes (as previously described) but failed to provide conclusive evidence for a causal gene variant.³¹ The previously reported pathogenic variants in the *PLP1* and *FNIP2* genes were not evident in the affected German Shepherds.

4 | DISCUSSION

We describe a novel hypomyelinating leukodystrophy in 11 German Shepherd dogs from 3 related litters. The 2-week-old dogs presented with a severe shaking puppy phenotype that interfered with the ability to be weaned and maintain themselves. We provide a detailed description of the long-term clinical course in 2 dogs and show that they walked with residual pelvic limb ataxia. Furthermore, we describe the imaging features of abnormal white matter maturation on repeated brain MRI.

Characteristic features of the shaking puppy syndrome are coarse tremors of the head and trunk evident soon after birth. Common causes are hypomyelination, dysmyelination, or spongiform leukoencephalopathies in dogs.^{3,13,14} Much information

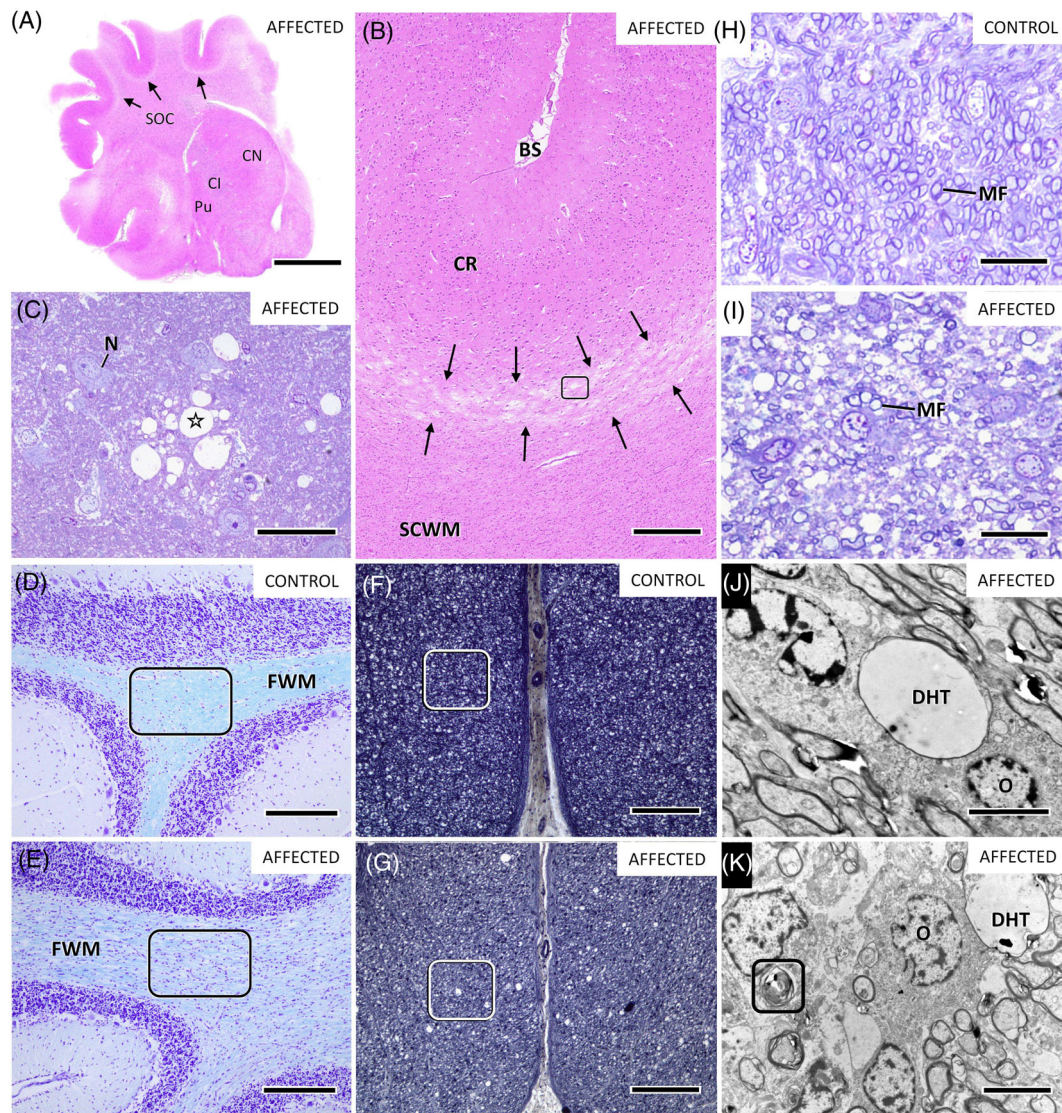


FIGURE 6 Representative neuropathological findings in frontal cortex (A-C), subcortical white matter (B, I, K), capsula interna (J), cerebellum (E), and thoracic spinal cord (G) of an affected dog (D, 7 weeks) and an age-matched control (D, F, H). In the frontal cortex, at subgross magnification, a pale line (A: arrows) signifies edema and spongiosis (B: black arrows) of inner cortical laminae and white-gray matter interface. Vacuolation of cortical neuropil (B: frame, C: asterisk) is a consequence to myelin splitting (not shown). Subcortical white matter (B: SCWM) including semioval center (A: SOC) stains abnormally pale and shows widespread neuroglial hypercellularity (B). Hypomyelination of central white matter becomes more evident using myelin specific dyes LFB (D, E) and WSS (F, G). Attenuation of myelin staining is demonstrated in foliary white matter (FWM) of cerebellum and in long spinal tracts of the affected dog (E, G) compared to the age-matched control (D, F); framed areas indicate the neuroglial white matter hypercellularity (E) and a mild white matter spongiosis of ventral funiculus (G). On semithin sections (H, I), myelinated fibers (MFs) in the affected dog (I) are sparse and present with variable calibers and degrees of myelination when compared to the control dog (H). Affected white matter shows microvacuolation (I) and contains numerous poorly myelinated fibers, dilated hypomyelinated myelin tubes (J, K: DHT) and degenerate myelin figures (K: frame). Oligodendrocytes appear hypertrophic (J, K: O) and show increased number and density (I). Stains and contrast agents: (A, B): Hematoxylin-Eosin (HE); (C, H, I): azure II methylene blue-safranin O (ASO); (D, E): Luxol fast blue-Cresyl echt violet (LFB); (F, G): Woelcke-Spielmeyer-Schröder stain (WSS); (J, K): uranyl acetate and lead citrate. Anatomical and histological labels: BS, base of sulcus; CI, capsula interna; CN, caudate nucleus; CR, cortical ribbon; FWM, foliary white matter; MF, myelinated fiber; N, neuron; O, oligodendrocyte; Pu, putamen; SCWM, subcortical white matter; SOC, semioval center. Magnification scale bars: (A): 0.5 cm; (B): 370 μ m; (C): 25 μ m; (D, E): 200 μ m; (F, G): 100 μ m; (H, I): 12.5 μ m; (J): 2500 nm; (K): 3000 nm

on the pathologic features of hypomyelination and dysmyelination is available in the veterinary literature.^{3,4,6,7,10} Nevertheless, pace and rate of recovery and whether affected dogs will be functional pets, which is of most interest to the owners and breeders, have not

always been documented well. Long-term observations of the German Shepherd dogs with the shaking puppy phenotype and hypomyelination described here indicated gradual but incomplete recovery with residual pelvic limb ataxia. Furthermore, epilepsy with

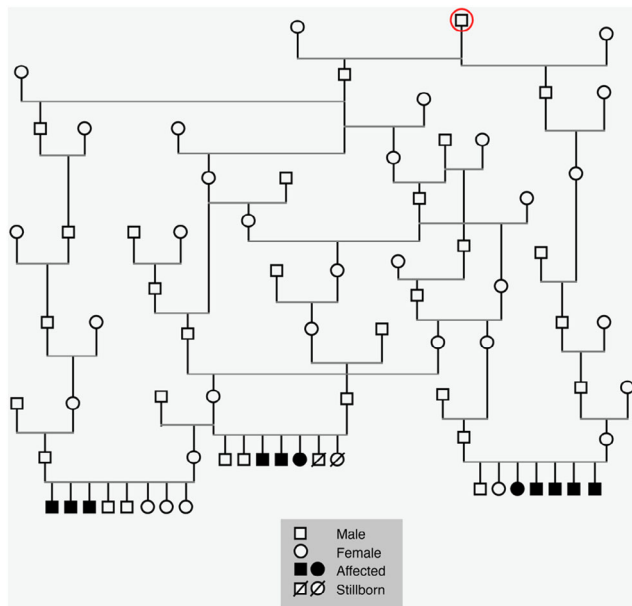


FIGURE 7 Pedigree of German shepherd dogs including two closely related German and one Finnish litter with multiple affected dogs. The parents of the affected litters share a common ancestor (red circle). The pedigree is consistent with an autosomal recessive mode of inheritance

focal motor and generalized epileptic seizures developed in 1 dog at 15 months of age. The veterinary literature commonly reports that dogs with shaking puppy syndrome may recover completely, but mild, moderate, or severe residual neurological signs also are possible. In summary, Weimaraners showed early recovery after 3 to 4 months of age with persistent fine tremor of the pelvic limbs or complete recovery by 1 year of age.^{7,11} In Chow-Chows, recovery plateaued later (between 6 and 8 months of age) with further gradual improvement and disappearance of neurologic signs at 12 months of age or with persistent mild intention tremor.¹⁰ Two male crossbred Lurcher puppies recovered completely by 16 weeks,⁹ generalized tremor in Bernese mountain dog puppies decreased with age,⁹ and recovery also was reported in cases in the Dalmatian, Golden Retriever, and a litter of Catahoulas.³² Samoyeds and Springer Spaniels develop severe clinical signs.^{4,33} Failure to recover and severe deficits occurred in male Springer Spaniels. Dogs remained blind, developed seizures, and showed uncontrolled whole-body movements, opisthotonus, and extensor rigidity of all limbs at 6 months of age. Only occasionally, some dogs showed some improvement. In contrast, some female Springer Spaniels were able to walk and showed tremor of variable severity, which disappeared over time.³² In the German Shepherd dogs, described here, no difference in the severity of clinical signs was noted between male and female dogs, even during long-term follow-up of female dogs. Affected female and male dogs were of the same size. There were more affected male dogs in all litters, which is probably just an effect of small numbers. The fact that females also were affected excludes X-linked inheritance. In the male Springer Spaniels, BAER recordings may be

abnormal, a common finding in humans with Pelizaeus Merzbacher disease (PMD) and abnormal auditory function.³³⁻³⁶ In contrast, BAER testing of 1 affected dog at 27 weeks of age failed to identify any abnormalities. Unfortunately, BAER testing was not done at an earlier age in the affected puppy. Conventional T2W brain MRI provided a diagnosis of generalized leukodystrophy. The entire subcortical white matter showed inappropriate homogenous T2W signal intensity (hyperintense, later isointense) until 27 weeks of age. This finding was consistent with delayed white matter maturation. Even the adult dog failed to display normal contrast between the gray and white matter: subcortical and cerebellar white matter appeared isointense or mildly hypointense when compared to gray matter, and the cerebellar arbor vitae was not visible. Brain images with T2W sequences and STIR images were most useful to identify abnormal white matter signal in the 7-week-old dogs, when compared to an unaffected littermate and previously described MRI features of brain maturation in Beagles.²⁶ In these, subcortical white matter displayed 3 phases of T2W brain maturation on transverse images: hyperintense appearance in the juvenile phase until 4 weeks of age, isointense in the T2W transition phase at 6 weeks of age, and hypointense in the maturing phase from 8 weeks to adulthood.²⁶ Cerebellar arbor vitae and brainstem already had an adult appearance at 6 weeks of age, and the corpus callosum was first seen at 6 weeks of age and had an adult appearance at 16 weeks of age on midsagittal T2W images.²⁶

Brain MRI showed homogenous hyperintensity of the entire subcortical white matter at 7 weeks of age on STIR images. However, STIR images at 27 weeks of age were as described in healthy Beagles. Therefore, the usage of STIR sequences at an early age may be helpful to characterize white matter disorders. Unfortunately, we failed to include STIR images at 15 weeks of age. In humans, STIR sequences are part of brain MRI protocols for evaluation of myelination in pediatric patients, mainly because of their ability to increase the gray-white matter contrast.³⁷ Conventional T1W-sequences were not useful in our dogs and failed to show a difference between the affected dogs and the unaffected littermate at 7 weeks of age. Signal intensity was comparable to T1W MRI of 8-week-old Beagles in all planes.²⁶ Beagles display progressive hyperintensity of subcortical white matter on T1W images from 6 weeks on, with first regional hyperintense signals evident in the T1W transition phase at 3 to 4 weeks of age.²⁵ The persistence of abnormal white matter signal on T2W-sequences in the adult dog (Figure 5) was in contrast to the resolution of tremors and shakes in the recovering dogs, but this finding correlated well with failure to recover normal gait and persistent severe pelvic limb ataxia in the adult dogs. Persistent abnormal myelination of the spinal cord white matter may have contributed to pelvic limb ataxia. Hypomyelination also was evident in the spinal cord of the necropsied dogs (puppies), but the spinal cord was not imaged in the adult dog. However, recognition of the abnormal white matter signal may be challenging without comparison to MRI of age-matched control dogs. In dogs, MRI features of shaking puppies were described in breeding colonies in the Springer Spaniel, but only advanced imaging with diffusion tensor and magnetization transfer and no conventional imaging

sequences were acquired.^{24,25} In humans, genetic diagnosis of leukodystrophies faces specific difficulties because of phenotypic heterogeneity and the fact that many patients with white matter disorders do not receive a final diagnosis. A multimodal approach, which considers specific MRI features, clinical signs, family history, and available genetic testing, is recommended.³⁸ Characteristic features of leukodystrophies in humans are the presence of T2W hyperintensity in the affected white matter, whereas T1W signal may be variable, compared to gray matter structures. A mildly hypo-, iso-, or hyperintense T1W signal suggests a hypomyelinating leukodystrophy whereas pronounced hypointense T1W signal has been considered more representative of demyelinating leukodystrophy.¹ The MRI features described here resemble the MRI pattern in X-linked recessive PMD in humans, which is the archetype of a hypomyelinating leukodystrophy. It also presents with homogenous prominent T2W hyperintensity and T1W hypointensity of cerebral white matter similar to what was observed in the dogs described here.^{19,39} Pattern recognition of MRI findings has evolved as a critical tool for definition of hypomyelinating leukodystrophies in humans. Repeated MRI images are recommended to distinguish primary hypomyelination from delayed myelination with respective variable etiologies.⁴⁰ Pattern recognition of MRI findings also has proven useful to define demyelinating leukodystrophies in veterinary medicine.^{17, 21–23, 41, 42}

We observed transient hyperammonemia which normalized over a few weeks in the absence of portosystemic shunting. In this context, we failed to identify decreased serum concentrations of cobalamin or methylmalonic aciduria.^{43,44} Both owners were heavy smokers, which could have contributed to false positive results of ammonia testing. Still, ammonia concentrations were only mildly increased in the unaffected littermate.⁴⁵ Transient hyperammonemia with normal bile acid concentrations may indicate urea cycle enzyme deficiencies, and has been described in Irish wolfhounds.⁴⁶ In humans, gene variants in *CPS1* underlying carbamoyl phosphate synthetase 1 deficiency are associated with severe neonatal hyperammonemia and leukodystrophy.⁴⁷ We cannot exclude a similar underlying process in the cases described here. However, no conclusive findings were identified on neurometabolic screening.

The puppies described here experienced intermittent saccadic oscillations and rapid, low amplitude mostly horizontal but also multidirectional aberrant involuntary eye movement with the absence of a drifting phase.⁴⁸ Abnormal eye movements also were described as nystagmus in Samoyeds, Springer Spaniels,⁶ Weimaraners,⁷ and Dalmatians²⁹ with shaking puppy syndrome and in dogs with inherited or acquired cerebellar disease.⁴⁹ In humans, the presence of elliptical pendular and upbeat nystagmus together with characteristic clinical history and MRI findings is indicative of PMD⁵⁰ but other hypomyelinating leukodystrophies with heterogeneous origin also may present with pendular nystagmus.⁵¹

Generally, there is no cure for hypomyelinating leukodystrophies in humans or dogs. A multidisciplinary approach is required in humans. In affected humans, treatment may include physical treatment with therapeutic exercises, antispasticity drugs (baclofen, diazepam, botulinum toxin), and eventually orthoses and anti-seizure drugs if seizures

occur.^{52,53} Involvement of gray matter may occur in genetic leukodystrophies, which could explain the origin of epileptic seizures later in life.⁵⁴ Idiopathic epilepsy is frequent in German Shepherd dogs,⁵⁵ but based on history and MRI features, we considered structural epilepsy related to the leukodystrophy as a likely cause. Pathologic examination of dogs from the 3 affected litters disclosed features of hypomyelination. Pathologic features, a clinical course with failure to recover completely, and MRI findings characterized the disease in the German Shepherd dogs as a hypomyelinating leukodystrophy. The early onset, uniformity of the clinical, MRI, and histopathologic findings suggest a hereditary basis for the disease similar to hypomyelination in Weimaraners¹¹ and Springer Spaniels.¹² However, we have not yet been able to identify the underlying genetic defect. Identification of the causative genetic variant will probably require analysis of additional affected dogs and could further define a natural disease model for leukodystrophies in humans and provide novel insights into pathophysiology and molecular mechanisms.^{11,56}

5 | CONCLUSION

We described a novel hypomyelinating leukodystrophy in 11 related German Shepherd dogs. The clinical course was nonprogressive and adult dogs walked with residual pelvic limb ataxia. Repeated conventional brain MRI was useful to characterize abnormal maturation of subcortical and cerebellar white matter. The disease is thought to be genetic in origin with an autosomal recessive mode of inheritance.

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CONFLICT OF INTEREST DECLARATION

Authors declare no conflict of interest.

OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

All diagnostic tests were performed following informed consent of the dogs' owners. The collection of blood samples was approved by the "Cantonal Committee for Animal Experiments" (Canton of Bern; permits 75/16 and 71/19) and ESAVI/7482/04.10.07/2015 (University of Helsinki).

HUMAN ETHICS APPROVAL DECLARATION

Authors declare human ethics approval was not needed for this study.

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